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Reduced Sensitivity to Fenarimol in Japanese Field Strains of *Venturia nashicola*

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Abstract: Monoconidial strains of *Venturia nashicola* Tanaka *et* Yamamoto were isolated from Japanese or Chinese white pear trees which had never been treated with sterol demethylation inhibitors (DMIs) and their baseline sensitivities to fenarimol were determined by mycelial growth tests on fungicide-amended culture media. Strains were also obtained from Japanese pear orchards, which had been intensively treated with DMIs for several years and monitored for the shifts of fenarimol sensitivity in comparison with the baseline sensitivity. Results suggested slight shifts to lower fenarimol sensitivity in strains isolated from DMI-treated Japanese pear orchards. However, in inoculation tests on pear seedlings, fenarimol still provided adequate control of *V. nashicola* strains with reduced sensitivity to fenarimol *in vitro*, suggesting that the performance of this fungicide will still be maintained in the field. © 1998 Society of Chemical Industry

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Key words: DMIs; fungicide resistance; baseline sensitivity; pear; fenarimol; *Venturia nashicola*

1 INTRODUCTION

Pear scab caused by *Venturia nashicola* Tanaka *et* Yamamoto is one of the most serious diseases of Japanese pear. Benzimidazole-resistant strains of *V. nashicola* are widely distributed throughout Japan, making it difficult to control the disease with benzimidazole fungicides.[1] Since 1986, sterol demethylation inhibitors (DMIs) such as fenarimol, bitertanol and triflumizole have been used in Japan for the control of this disease. In general, the risk of DMI resistance in the field is considered to be lower than that of resistance to benzimidazole fungicides.[2] It has also been shown that, in *V. nashicola* populations, shifts to lower DMI sensitivity are unlikely to proceed very quickly.[3] However, in other countries, strains resistant to DMIs have already been detected in natural populations of some phytopathogenic fungi including *V. inaequalis*,[4] *Penicillium digitatum* (Pers.) Sacc.[5] and *Uncinula necator* (Schw.) Burr.[6] Furthermore, in some Japanese pear-growing areas, DMIs have been intensively used up to seven to 10 times per season, although these fungicides were recommended for use no more than three times a year. In this paper, the authors first report the appearance of field strains of *V. nashicola* that carry reduced sensitivity to fenarimol.

2 EXPERIMENTAL METHODS

2.1 Fungal strains

For monitoring fenarimol sensitivity, between 1991 and 1995, pear leaves with sporulating scab lesions were collected from commercial orchards in Ibaraki, Saga, Oita, Fukuoka, Okayama, Tottori and Hiroshima Prefectures, Japan, where DMIs had been frequently applied. To obtain the baseline sensitivity data, samples of pear

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leaves were also taken from one tree in a house garden, Iwama, Ibaraki, Japan in 1992, and from one orchard in Hebei, People's Republic of China in 1993. DMIs had never been applied at either of these sites. (We were permitted to use the latter *V. nashicola* strain by Plant Protection Station, MAFF, Japan.)

In our preliminary experiments, two monoconidial strains for each were obtained from nine lesions naturally formed on DMI-treated Japanese pear leaves. The fenarimol sensitivities of strains isolated from a single lesion often differed markedly in their EC_{50} values e.g. 0·7 to 0·8 versus 0·1 to 0·2 mg litre^{-1}. This means that fungal strains derived from the same lesion do not necessarily belong to the same clone. Therefore, we judged it essential to use monoconidial strains for the DMI sensitivity monitoring of *V. nashicola.*

Conidia collected from lesions were suspended in sterile distilled water. Drops of conidial suspensions were placed on water agar plates and incubated at 15°C for three to five days. Agar blocks containing one germinated conidium were individually cut with a steel needle after microscopic observation and transferred onto potato-dextrose agar (PDA) slants amended with penicillin G potassium salt (50 mg litre^{-1}), streptomycin sulfate (50 mg litre^{-1}) and lactic acid (0·6 g litre^{-1}). After incubation at 20°C, monoconidial cultures of *V. nashicola* were obtained and used for tests of fenarimol sensitivity.

2.2 Fungicides and fenarimol sensitivity tests

A commercial 120 g kg^{-1} fenarimol WP was used. Each isolate was cultured on PDA plates at 20°C for 60 days to supply inoculum. Mycelial discs, 4 mm in diameter, were cut from colony margins and transferred onto PDA plates containing 0, 0·01, 0·05, 0·1, 0·5, 1, 5, 10, 50 and 100 mg AI litre^{-1} of fenarimol. Fenarimol suspended in distilled water was added aseptically to molten PDA after autoclaving. After incubation at 20°C in the dark for three weeks, the diameter of each colony was measured and the EC_{50} for fenarimol was calculated using a log-linear model software kindly supplied by ZEN-NOH (Tokyo).

2.3 Inoculation tests

In 1993, leaves with sporulating scab lesions were randomly collected from the pear orchards, Yasato 1 and Yasato 2, Ibaraki Prefecture and Imari 1, Imari 3, Saga Prefecture where strains with lower fenarimol sensitivity had occasionally been detected in populations of *V. nashicola* during the previous year, 1992. Conidia were washed from lesions with distilled water by centrifugation and directly used for inoculation tests. As a reference, conidia were collected from one tree in a house garden in Iwama, Ibaraki, which had never been treated with DMIs.

Monoconidial strains of *V. nashicola* showing reduced sensitivity to fenarimol (EC_{50} > 1 mg litre^{-1}), which had been isolated from Imari 1, Imari 3, Saga and Iwama, Ibaraki in 1992, were selected. In 1993, conidia of these strains and a reference sensitive strain were produced on a culture medium and also used for inoculation tests. Methods for conidial formation will be reported in detail elsewhere. Conidia were suspended in sucrose solution (1 g litre^{-1}) and the suspension adjusted at 1×10^4–1×10^5 conidia ml^{-1}. Young leaves of pear seedlings were sprayed with these conidial suspensions. Inoculated seedlings were kept at 20°C in a dew chamber for 48 h. After drying, the inoculated seedlings were sprayed with fenarimol suspensions at different concentrations and kept in a phytotron at 25°C (day)/20°C (night). One month after inoculation, the number of scab-sporulating leaves was assessed.

3 RESULTS

3.1 Baseline sensitivity to fenarimol

EC_{50} values of fenarimol for 46 strains isolated from 27 lesions on a pear tree which had never been treated with DMIs in Iwama, Ibaraki, Japan, ranged from 0·02 to 1·29 mg litre^{-1} and the average was 0·199 mg litre^{-1}

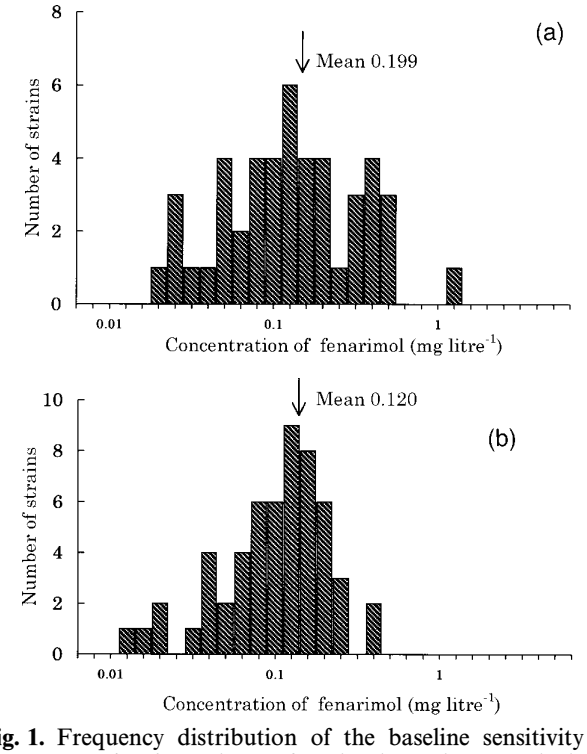

Fig. 1. Frequency distribution of the baseline sensitivity of *Venturia nashicola* strains to fenarimol. Strains were isolated from (a) one tree in a house garden, Iwama, Japan and (b) one orchard in Hebei, People's Republic of China. Isolation of the fungus was done in 1992 and 1993 respectively when DMIs had never been applied to either sites. The data are based on EC_{50} values of fenarimol for mycelial growth of each strain.

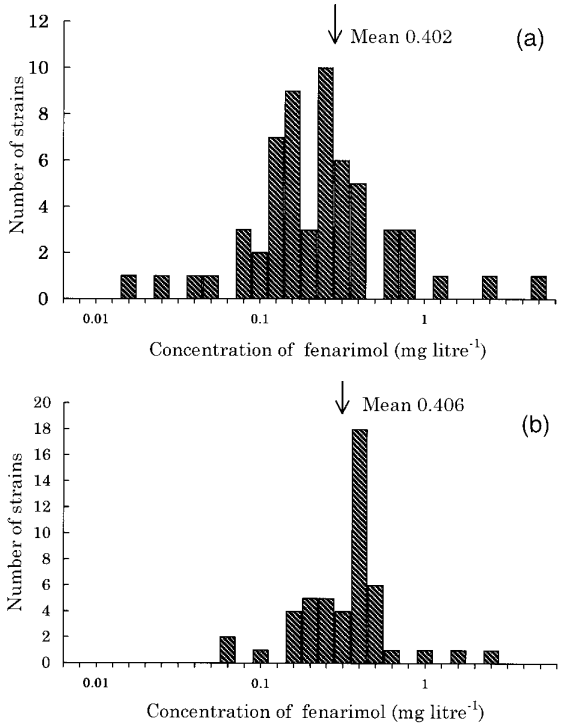

Fig. 2. Frequency distribution of the sensitivity of *Venturia nashicola* strains to fenarimol. Strains were isolated from (a) trees in the commercial orchard Yasato 2 and (b) Amagi 1 where DMIs had routinely been applied for several years. Isolation of the fungus was done in 1992 and 1993 respectively. The data are based on EC_{50} values of fenarimol for mycelial growth of each strain.

(Fig. 1a). For only one strain was the EC_{50} value higher than 1 mg litre^{-1}. Moreover, for 53 strains from 21 lesions collected in Hebei, People's Republic of China, EC_{50} values of fenarimol ranged between 0·01 and 0·45 mg litre^{-1}, and the average was 0·120 mg litre^{-1} (Fig. 1b).

3.2 Monitoring for fenarimol sensitivity

In 1992, three orchards in Saga and two in Ibaraki were monitored (Table 1). In some orchards (Fig. 2a), slight shifts in fenarimol sensitivity were observed in fungal populations and strains with reduced sensitivity were occasionally detected. Four different orchards were monitored in Saga in 1993 and 1994, together with two in Fukuoka in 1993. Strains with reduced sensitivity to fenarimol were again found in some orchards (Fig. 2b), but their frequency still remained low. Mean EC_{50} values determined for individual orchard populations were compared according to the nonparametric Kolmogorov–Smirnov test. As a whole, only one population was statistically significant from the others (Table 1).

To simplify the monitoring experiments, the relative mycelial growth of individual strains at a single fenarimol concentration (0·5 mg litre^{-1}) was calculated (Fig. 3). The relative mycelial growth of all strains isolated from non-DMI-treated trees was less than 50% of the

TABLE 1
Sensitivity of Field Strains of *Venturia nashicola* to Fenarimol

Year	*Prefecture*	*Orchard*	*Number of strains tested*	*Range of EC_{50} (mg litre^{-1})*	*Mean EC_{50} (mg litre^{-1})*	P(*Mean EC_{50}*)[a]
1992	Ibaraki	Yasato-1	49	0·02 ~ 0·92	0·16	ns[c]
		Yasato-2	58	0·02 ~ 2·78	0·40	ns
	Saga	Imari-1	60	0·01 ~ 3·19	0·36	ns
		Imari-3	46	0·01 ~ 3·38	0·31	ns
		Imari 14	44	0·03 ~ 0·65	0·20	ns
1993	Saga	Imari 1	50	0·01 ~ 0·68	0·14	ns
		Imari 3	14	0·11 ~ 3·42	0·85	s[d]
		Imari 5	13	0·07 ~ 1·71	0·48	ns
		Imari 6	11	0·05 ~ 0·39	0·19	ns
	Fukuoka	Amagi 1	49	0·06 ~ 1·72	0·41	ns
		Amagi 2	19	0·06 ~ 1·03	0·28	ns
1994	Saga	Imari 1	50	0·01 ~ 3·66	0·36	ns
		Imari 3	47	0·01 ~ 0·79	0·17	ns
		Imari 5	33	0·02 ~ 1·13	0·25	ns
		Imari 6	50	0·01 ~ 1·39	0·23	ns
Reference[b]						
1992	Ibaraki	Iwama	46	0·02 ~ 1·29	0·20	ns
1993	Hebei (China)		53	0·01 ~ 0·45	0·12	ns

[a] Smirnov test.
[b] Pear trees had never been treated with DMIs.
[c] Not significant.
[d] Significant ($P = 0·05$).

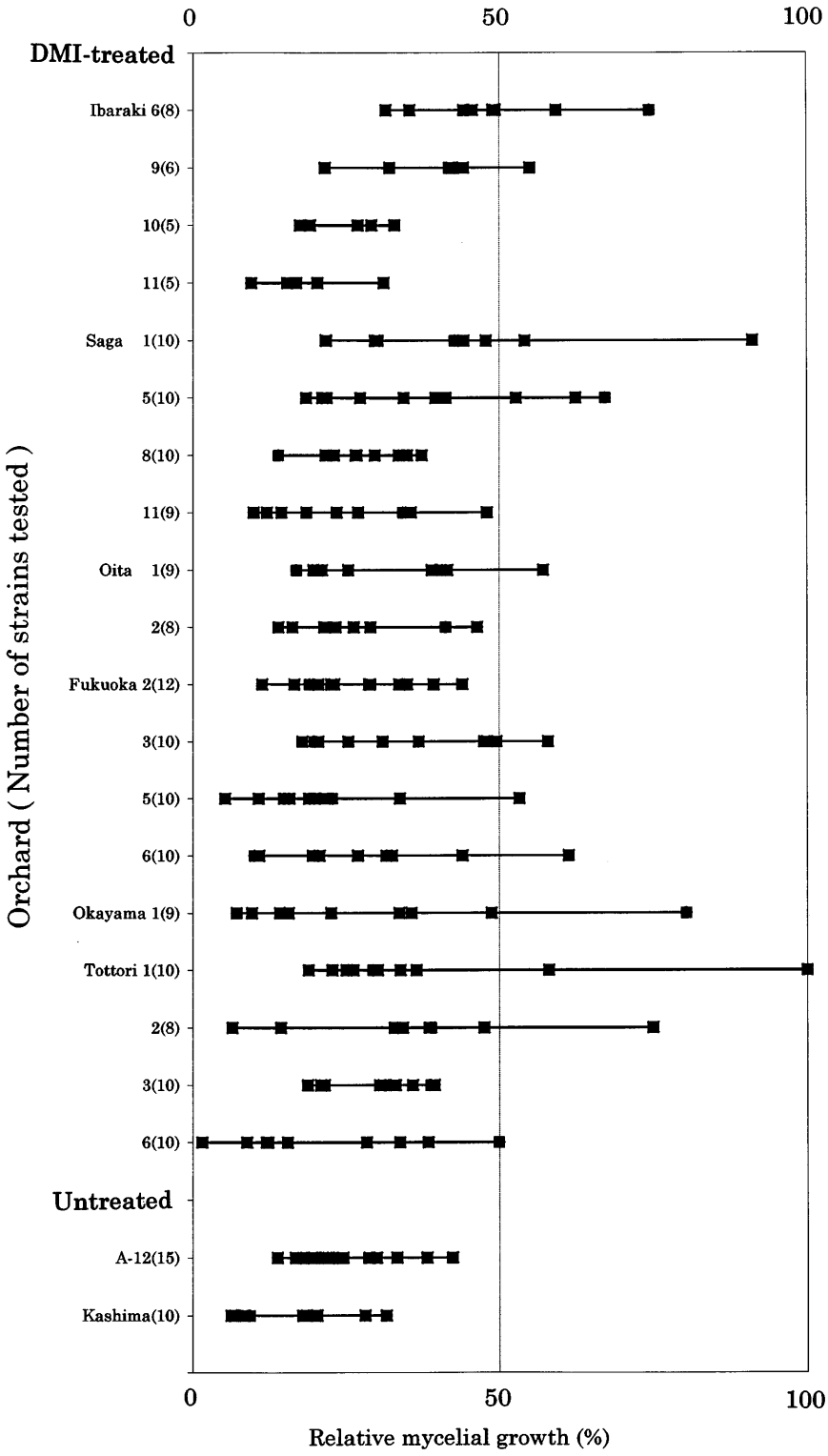

Fig. 3. Sensitivity of *Venturia nashicola* strains to fenarimol at 0·5 mg litre^{-1}. Strains were isolated from DMI-treated orchards and untreated trees in 1991. Relative mycelial growth is defined as the linear growth on agar medium amended with 0·5 mg fenarimol litre^{-1}, expressed as a percentage of the growth on unamended medium. Symbols in the figure represents the relative mycelial growth of each strain.

growth on a fungicide-free control medium. However, several strains isolated in 1991 from DMI-treated orchards in Ibaraki, Saga, Oita, Fukuoka, Okayama and Tottori showed mycelial growth higher than 50% of the control.

3.3 Inoculation tests

Conidia collected from leaves from orchards in which the fenarimol sensitivity of fungal populations had slightly decreased were used for the tests. Conidia

obtained from leaves from one pear tree in a home garden, Iwama, which had no history of the use of DMIs, were used as a control. In both cases, spray applications of fenarimol effectively controlled pear scab even at half the concentration recommended for use in orchards (Table 2). Conidia from monoconidial strains less sensitive to fenarimol were also used as inoculum, but fenarimol adequately controlled these strains as well as a reference strain (Table 3).

4 DISCUSSION

Since 1986, DMIs have been used in Japan for the control of pear scab, but shifts to lower DMI sensitivity were not likely to proceed very quickly in this fungus.[3] To avoid potential problems of DMI resistance and maintain the long-term effectiveness of DMI fungicides, their use has been recommended to a maximum of three times during a pear-growing season. In some areas, however, DMIs have been intensively used from seven to 10 times per season.

No obvious signs have yet been seen of the failure of DMIs to control pear and apple scab in Japan.[7] On the other hand, in a field test carried out in Canada, a reduction in apple scab control was first observed six years after DMIs were introduced and used seven or nine times per season. After that, the levels of resistance increased over the next three years, and eventually disease control failed completely.[8] The failure of DMIs to control apple scab has also been reported from commercial orchards in Italy[9] and Austria.[10] Consequently, we monitored fenarimol sensitivity in field populations of *V. nashicola* in several Japanese Prefectures. When we started the monitoring work, it was hard to find Japanese pear orchards which had never been exposed to DMIs, as all farmers so far contacted had routinely been using this group of fungicides. To obtain the baseline sensitivity data needed, we tested 99 strains in total, which were isolated from a Chinese white pear orchard in the People's Republic of China and one pear tree in a house garden in Japan where DMIs had never been used (Wang Wenqiao, pers. commun., 1993).

The frequency distributions of fenarimol sensitivity were not significantly different in the two fungal populations in China and Japan (Table 1), despite a geographic separation of over 2000 km. In these baseline sensitivity tests, average EC_{50} values of fenarimol sensitivity were 0·120 and 0·199 mg litre^{-1} respectively. Based on these sensitivity distributions (Fig. 1), strains with EC_{50} values in excess of 1·0 mg litre^{-1} were considered to be less-sensitive strains. The baseline sensitivity of *V. inaequalis* strains expressed as the EC_{50} value of fenarimol for growth of mycelia was 0·04 mg

TABLE 2
Effects of Fenarimol on the Incidence of Pear Scab

Orchard from which conidia were collected and used as inocula		*Control (%)*[a]	
		Fenarimol (mg litre^{-1})	
		30[b]	*15*
Ibaraki	Yasato 1	97·3	89·2
	Yasato 2	100	88·7
Saga	Imari 1	80·7	100
	Imari 3	95·3	100
Ibaraki	Iwama (Reference)	100	92·8

[a] Values do not differ significantly (Tukey test) ($P = 0·05$).
[b] Concentration recommended for use in orchards.

TABLE 3
Effects of Fenarimol on the Incidence of Pear Scab

Strain inoculated		*EC_{50} of fenarimol (mg litre^{-1})*	*Control (%)*[a]	
			Fenarimol (mg litre^{-})	
			30[b]	*15*
Saga	Imari 1-29-2	3·194	100	100
	Imari 1-44-1	2·705	100	85
	Imari 3-29-1	3·375	100	100
Ibaraki	Iwama 1-1	1·286	94·8	70·6

[a] Values do not differ significantly (Tukey test) ($P = 0·05$).
[b] Concentration recommended for use in orchards.

litre^{-1}.[11] Thus the inherent sensitivity to fenarimol was three to five times lower in *V. nashicola* as compared with that of *V. inaequalis*.

Monoconidial strains of *V. nashicola* were isolated from orchards which had been intensively treated with DMIs for several years and monitored for any shift in fenarimol sensitivity. In 1992, small shifts in fenarimol sensitivity were observed in fungal populations and less-sensitive strains were occasionally detected in some orchards (e.g. Yasato 2). In the following year, fungal strains with reduced sensitivity to fenarimol were also found in other orchards (e.g. Amagi 1). In 1994, the detection of less-sensitive strains in orchards still remained at a frequency between 3·0 and 6·0% of the total population. In 1995, 56 monoconidial fungal strains were isolated from an orchard in Saeki, Hiroshima, where heavy attack of pear scab had been observed the previous year despite intensive use of DMIs for scab control (approximately 40 applications in total between 1991 and 1994). The average EC_{50} value, 0·218 mg litre^{-1} for fenarimol, was only slightly higher in fungal populations as compared with the baseline sensitivity, and EC_{50} values of all strains never exceeded 1 mg litre^{-1} (range: 0·05–0·70 mg litre^{-1}). Field spray-application trials subsequently conducted in 1997 have shown that adequate performance of DMIs has still been maintained in this orchard (H. Nitta, pers. comm.).

When DMI sensitivity is examined in mycelial growth tests, comparisons are based on EC_{50} rather than MIC values in general.[3] In our monitoring experiments, nine fenarimol concentrations were regularly used, but this method is extremely laborious and time-consuming. To reduce labour and time, the example of Köller *et al.*[11] was followed, as relative growth values were correlated with EC_{50} values in *V. inaequalis*.[12] Relative mycelial growth of individual strains was assessed at a single discriminating concentration (0·5 mg litre^{-1}). Strains with lower fenarimol sensitivity were found from many well-separated commercial orchards (Fig. 3). Whilst shifts to lower fenarimol sensitivity were observed in field populations of *V. nashicola* in Japan, there was no evidence that the efficacy of DMIs for scab control declined in pear orchards.

Finally, spore samples collected from commercial orchards, or strains which showed reduced sensitivity to fenarimol *in vitro*, were used for inoculation tests on pear seedlings to examine whether efficacy of fenarimol was reduced by these strains. Fenarimol provided adequate control of *V. nashicola* strains with reduced sensitivity *in vitro*, suggesting that the performance of fenarimol was still maintained in the field. However, to avoid potential problems on DMI resistance, it is still necessary to reduce disease pressure and to keep the frequency of applications as low as possible. Currently, DMIs are recommended for use up to three times per year in pear orchards.

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REFERENCES

1. Ishii, H., van Raak, M., Inoue, I. & Tomikawa, A., Limitations in the exploitation of *N*-phenylcarbamates and *N*-phenylformamidoximes to control benzimidazole-resistant *Venturia nashicola* on Japanese pear. *Plant Pathol.*, **41** (1992) 543–53.
2. Köller, W. & Scheinpflag, H., Fungal resistance to sterol biosynthesis inhibitors. *Plant Dis.*, **71** (1987) 1066–74.
3. Ishii, H., Takeda, H., Nagamatsu, Y. & Nakashima, H., Sensitivity of the pear scab fungus (*Venturia nashicola*) to three ergosterol-biosynthesis-inhibiting fungicides. *Pestic. Sci.*, **30** (1990) 405–13.
4. Thind, T. S., Clerjeau, M. & Olivier, J. M., First observations on resistance in *Venturia inaequalis* and *Guignardia bidwellii* to ergosterol-biosynthesis inhibitors in France. *Proc. 1986 Brit. Crop Prot. Conf.*, **2** (1986) 491–8.
5. Eckert, J. W., Sievert, J. R. & Ratnayake, M., Reduction of imazalil effectiveness against citrus green mold in California packinghouses by resistant biotypes of *Penicillium digitatum*. *Plant Dis.*, **78** (1994) 971–4.
6. Gubler, W. D., Ypema, H. L., Ouimette, D. G. & Bettiga, L. J., Occurrence of resistance in *Uncinula necator* to triadimefon, myclobutanil, and fenarimol in California grapevines. *Plant Dis.*, **80** (1996) 902–9.
7. Ishii, H., Resistance management in diseases of top fruit in Japan. *Pestic. Sci.*, **51** (1997) 383–6.
8. Hildebrand, P. D., Lockhart, C. L., Newbery, R. J. & Ross, R. G., Resistance of *Venturia inaequalis* to bitertanol and other demethylation-inhibiting fungicides. *Can. J. Plant Pathol.*, **10** (1988) 311–16.
9. Fiaccadori, R., Gielink, A. J. & Dekker, J., Sensitivity to inhibitors of sterol biosynthesis in isolates of *Venturia inaequalis* from Italian and Dutch orchards. *Neth. J. Pl. Path.*, **93** (1987) 285–7.
10. Hermann, M., Szith, R. & Zinkernagel, V., Verringerte sensitivität einiger isolate von *Venturia inaequalis* aus der

Steiermark für EBI-fungizide. *Gartenbauwissenschaft*, **54** (1989) 160–5.
11. Köller, W., Parker, D. M. & Reynolds, K. L., Baseline sensitivities of *Venturia inaequalis* to sterol demethylation inhibitors. *Plant. Dis.*, **75** (1991) 726–8.
12. Köller, W., Wilcox, W. F., Barnard, J., Jones, A. L. & Braun, P. G., Detection and quantification of resistance of *Venturia inaequalis* to sterol demethylation inhibitors. *Phytopathology* **87** (1997) 184–90.